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Chemosignalling effects of human tears revisited: Does exposure to female tears decrease males' perception of female sexual attractiveness?

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ABSTRACT

Gelstein et al. reported the results of three experiments suggesting a dampening influence of inhalation of female emotional tears on males' arousal and perception of female sexual attractiveness, specifically in non-sexual situations. This prompted the hypothesis that crying exerts its influence on others not only via the auditory and visual mode but also via chemosignals. In three studies, we attempted to replicate and extend Gelstein et al.'s findings by including an additional condition with irritant tears, by using pictures of sexually attractive women, and by testing related hypotheses on the pro-social effects of exposure to tears. All three studies, separately or combined in a meta-analysis, failed to replicate the original inhibitory effects of tears. In addition, sniffing tears did not affect measures of connectedness, aggression and pro-social behaviour. It is concluded that the effects of female tears on male arousal and perception of female sexual attractiveness, if any, are very weak at best. Rather, it seems that crying exerts its strong inter-personal effects through the visual and auditory sensory channels.

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The functions of human crying are supposed to be found in the intra-personal and inter-personal domain (e.g. Cornelius, 2001; Vingerhoets & Bylsma, 2015). Regarding the intra-personal domain, there is a broad literature, in particular in the popular media, which claims that crying results in catharsis, that is, that it reduces tension and improves mood. However, as made clear by Rottenberg, Bylsma, and Vingerhoets (2008), these beneficial effects are certainly not a fixed result of crying. Rather, how a crier feels after a crying episode seems to depend on characteristics of the crier, on the specific crying antecedent, and on how others respond to the crying. The possible underlying mechanisms are still far from being clarified and are proposed to be of a physiological, cognitive as well as social nature (Gračanin, Bylsma, & Vingerhoets, 2014).

In the inter-personal domain, tears can be considered as a signal that conveys information about the helplessness of the crying individual (Hasson, 2009; Vingerhoets & Bylsma, 2015), resulting in increased motivation of observers to react with pro-social behaviour (Vingerhoets, van de Ven, & van der Velden, 2016). However, the inter-personal effects of crying are also not always consistent. Although evidence suggests that the perception of tears generally results in helping behaviours, strengthening of social bonds and a reduction of aggression (see below), there is convincing anecdotal evidence that (particularly acoustical) crying may also sometimes evoke irritation and even aggression and violence (see Alexander, 2003). The precise determinants of the reactions of others to crying still wait to be disclosed. Nevertheless, it seems obvious that the personality of

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the observer, the specific antecedent and the perceived appropriateness of crying, as well as the relationship between crier and observer may all play a role. In addition, how the crier precisely expresses his/her distress may be a crucial factor (see Vingerhoets, 2013).

Until now, studies on the effects of exposure to crying individuals mostly focus on the auditory aspects (acoustic characteristics of crying) or on the visual component (visibility of tears). The effects of acoustical crying have been studied solely in infants (e.g. Crowe & Zeskind, 1992; Joosen et al., 2012; Riem, Voorthuis, Bakermans-Kranenburg, & Van Ijzen-doorn, 2014), whereas the role of visible tears has been studied mainly in adults (e.g. Balsters, Krahmer, Swerts, & Vingerhoets, 2013; Cornelius & Lubliner, 2003; Cornelius, Nussbaum, Warner, & Moeller, 2000; Hendriks & Vingerhoets, 2006; Provine, Krosnowski, & Brocato, 2009; but see also Zeifman & Brown, 2011). This line of research yielded considerable evidence that tears promote pro-social responses in observers, which was also found to be mediated by cognitive processes such as attributions of positive traits to tearful individuals (e.g. Vingerhoets et al., 2016).

Recently, Gelstein et al. (2011) reported a series of three within-subjects studies suggesting that crying might produce its inter-personal effects also via a third pathway: the presence of certain chemosignals in tears. According to these authors, tears contain a biochemical substance (pheromone?) that might also affect the behaviour and psychobiological functioning of others in the near environment of the crier. Their first, double-blind study showed that, in 24 male study participants, sniffing freshly harvested tears from three donor women who had watched sad movies, resulted in reduced ratings of the sexual attractiveness of women's faces (WF), compared to when participants inhaled saline. In the second study, the tears collected from five female donors resulted, in comparison to saline, in a greater decrease in sexual arousal in response to a *sad* film, in a dampened autonomous physiological activation, as well as in reduced levels of plasma testosterone, in 50 male participants. Finally, in the third study, exposure of 16 male participants to the donor tears reduced the activity of brain structures associated with sexual arousal, when watching sad, happy, or neutral movies.

Gelstein et al.'s findings and conclusions can in our opinion be criticised on at least two grounds. First, they did not conduct the appropriate test in the second study; rather than comparing if a statistically

significant difference is present in the tears and not in the saline condition, a test of the interaction effect would have been more appropriate. Carrying out this interaction test using the statistics reported in Gelstein et al. yields no evidence of tears versus saline ($d = .17$, $t(49) = 1.21$, $p = .23$).¹ Second, Gelstein et al. (2011) did not study the effects of tears on the sexual attraction and arousal induced by erotic stimuli; they explicitly stated that "... tears consistently reduced sexual arousal in men in a non-sexual setting (viewing pictures of faces and sad or neutral films)" (Gelstein et al., 2011, p. 230). On the other hand, the effect of tears on testosterone levels has been replicated in a study by Oh, Kim, Park, and Cho (2012). In addition, Ferrero et al. (2013) demonstrated that juvenile mice secrete a substance from their lacrimal gland, labelled exocrine gland-secreting peptide 22 (ESP22), which inhibits sexual behaviour in adult mice and prevents pedophilic assaults. While some caution is needed when generalising this kind of observations in rodents to humans, this study nevertheless suggests that, at least in some other species, such a mechanism may exist. Therefore, there is some support for the notion that chemosignalling effects of tears exist in humans, although for such a conclusion a more focused research is necessary.

The present report addresses three attempts to replicate and extend the Gelstein et al. (2011) findings, although with some important adaptations. Following up on Gelstein et al., in each of the studies, we evaluated the effects of fresh female tears on the ratings of pictures of women by male study participants. The first two studies were based on a between-subjects design, implying the exposure of different participants to different substances. In the second study, we additionally implemented a mixed design that included pre- and post-inhalation measurements. The third study was based on a within-subjects design and followed exactly the same procedure as the Gelstein et al. (2011) study. The main extensions concerned the following aspects. First, we not only compared emotional tears and saline, as was done by both Gelstein et al. (2011) and Oh et al. (2012), but we added a third condition, in which the participants were exposed to irritant tears of the same tears donors. Second, in the first two studies we used pictures of pre-selected sexually attractive women, including not only faces but also the pictures of the whole women bodies as stimuli. Third, given the replicated effect on testosterone and the established links between testosterone and aggressive behaviour

(e.g. Book, Starzyk, & Quinsey, 2001), we hypothesised that not only sexual attraction but also aggression might be reduced. Consequently, we also evaluated the effects of sniffing tears on measures of aggression. Finally, we hypothesised that the inhalation of tears might promote pro-social behaviour and feelings of social connectedness (e.g. Hasson, 2009; Vingerhoets, 2013). When designing and conducting each of the three studies, we made sure that the experimenters were not aware of the results of the other two studies. Where appropriate, we report how we determined our sample size, all data exclusions, all manipulations, and all measures in each study.

Study 1

In Study 1, the male participants rated the sexual attractiveness and some psychological traits of women presented in a series of pictures. The ratings of psychological, socially desirable traits allowed us to control statistically for possible response set tendencies and other factors that may systematically influence individual participants' answers. Relatedly, we measured participants' mood at the beginning of the experiment, to be able to control statistically for its impact on the dependent variables. In addition to the ratings of pictures of WF that were addressed in the Gelstein et al. (2011) study, we additionally used pictures of semi-nude women, showing their whole bodies as stimuli. For the evaluation of possible effects of saline or tears on pro-social tendencies, we measured aggressiveness, generosity, and social connectedness. Finally, in order to control for the possibility that the emotional state experienced by the donors during the production of emotional or irritant tears might moderate the effects on the dependent measures, we also measured the donors' emotions during the tears collection.

Method

Participants and design

We included 75 students in our study, 25 in each condition, to have at least as many participants in each condition as Gelstein et al. (2011) had in their first study. All students were male (age range 18–34; $M = 22.88$, $SD = 2.92$) and received course credits for participation. Six participants reported suffering from cold sores on their nose, but given the reported ability to sense odours, still were included in the

sample. The study used a double-blind between-group design (25 participants per group), in which participants were randomly assigned to one of the three conditions: (1) emotional tears; (2) irritant tears; and (3) saline. Both the participants and the experimenter who exposed them to the content of the test tubes containing one of the three types of substances were blind with respect to the administered substance.

Stimuli

The initial picture set included 40 pictures of highly attractive women in lingerie or bikini (WB) and 20 pictures of attractive WF collected from various internet sources. All the pictures were rated for attractiveness in a pilot study by 13 male participants, on a 10 points scale. Six pictures of WF and six pictures of WB with the highest attractiveness scores (WF: average $M = 6.51$; average $SD = 1.95$; WB: average $M = 7.22$, average $SD = 2.30$) were selected for the main study. None of the depicted women were known to the experimenters and participants from both the pilot and the main study.

Tears collection

We followed the procedure for tear collection and exposure as described by Gelstein et al. (2011) as accurately as possible. During the harvesting of tears, the donors did not wear make-up, and their face was cleansed at the start of each session. The emotional tears were elicited by sad films (e.g. Hachi: A dog's Tale; Hallström, 2009), and were harvested by the three donors themselves. The donors were psychology students (age 20–21 years) participating in the study on a voluntary basis. Before the application of the saline, it was first trickled down the cheeks of the donors to control for the possible contaminating effects of any skin-bound odour sources or other possible bioactive substances. Irritant or reflex tears were evoked using the Kryolan® tear blower, which consists of an acrylic glass holder filled with menthol crystals. The menthol vapours that are blown into the donor's eyes generally immediately produce tears. The donors used sterilised 35 mm long test tubes for the collection of both tears and saline. The first experimenter wore disposable gloves when transporting the closed test tubes to the participants' room. The period during which the compound was kept in test tubes before the application varied between 5 and 30 min.

Procedure

Each participant signed informed consent, answered questions about being a smoker (yes/no), and having a runny nose (yes/no), and completed the *friendly and peaceful mood* (1st measurement) and *general mood* scales, all in a separate room, before he was accompanied by the second experimenter to the laboratory room and individually seated approximately 60 cm from a 19-inch monitor. The participants were instructed to sniff deeply the tears (or saline) in the test tube three times, and, subsequently, the compound (around 100 µl) was deposited onto pads pasted to their upper lip, under the nostrils, to ascertain a continuous exposure during the tasks.

After the experimenter left the room, the participant received the instructions on the screen, which were followed by a presentation of the 12 pictures (6 WF followed by 6 WB) and short time intervals for rating each of them, then by the instructions for the *dictator game*, for how to answer the *social connectedness* question and the *friendly and peaceful mood* scale (2nd measurement), with enough time for each of the tasks after a particular instruction. All stimuli and instructions were presented in time sequences of the same length for each participant. The complete procedure before the second assessment of *friendly and peaceful mood* lasted eight minutes.

Measures

Each picture was rated on eight items, on a 10-points scale. The following four items were directly relevant to our main hypothesis and were used to create the major dependent variable *sexual attractiveness*: (1–2) *How sexy/attractive is the depicted woman?* (3) *Suppose you were single, would you like to date the depicted woman?* and (4) *To what extent does the depicted woman arouse you?* The additional four items (*How intelligent/reliable/caring/emotionally stable is the depicted woman?*) were used to measure *psychological/socially desirable traits*. Ratings on each of the four items measuring different aspects of attractiveness were averaged across six WF pictures, yielding four average item scores. Next, a composite variable was created from these four averages and labelled as *WF Attractiveness*. Variables *WF Socially Desirable Traits*, *WB Attractiveness*, and *WB Socially Desirable Traits* were created using the same procedure. Cronbach alphas were 0.95 (WB) and 0.94

(WF) for sexual attractiveness, and 0.90 (WB) and 0.89 (WF) for socially desirable traits.

Friendly and peaceful mood was measured with eight items, using a 100 mm visual analog scale (VAS, e.g. *amiable, peaceful, friendly*) with anchor points *I do not feel that way at all* and *I completely feel that way* written at the extremities. Cronbach alphas were 0.83 and 0.84 for measurements 1 and 2, respectively. The variable *Friendly and Peaceful Mood change* was computed by subtracting this scale's score before the experiment from its score measured after the inhalation of the compound.

Generosity, as an index of pro-social behaviour, was operationalised as the result of a dictator game (e.g. Edele, Dziobek, & Keller, 2013). Each participant had to decide about the share of money of 100 units of national currency that he would donate to a (non-existing) participant in another room. It was explicitly stated that participants were playing a game and that no real money was shared.

For the measurement of *social connectedness*, we applied the *Inclusion of Other in the Self Scale* (IOS, Aron, Aron, & Smollan, 1992). This single-item pictorial measure consists of a set of seven increasingly overlapping circle pairs, which represent, respectively, the participant himself and others. The participant's task was to choose the combination of circles that best represented his level of *connectedness* to other people.

General mood at the beginning of the experiment was measured by the *Emotional States Scale* (Kardum & Bezinović, 1992) containing 40 items (e.g. *depressed, lively*) on a 5-point Likert scale ranging from 1 (*I do not feel this way at all*) to 5 (*I feel this way completely*). The scale had adequate internal consistency, with the Cronbach alphas for Positive Affect (PA) (20 items) and negative affect (NA) (20 items) scale of 0.71 and 0.93, respectively.

The emotional state of the donors during the tear collection procedure was assessed by 11 items (e.g. *sorrow, pain, being tense*) on a 5-points Likert scale, immediately after the tear collection was finished (Cronbach alpha = 0.75).

Results

None of the potentially confounding variables (donor 1, 2 or 3 and participants' age) were significantly related to the ratings of perceived sexual attractiveness. A significant difference in negative emotional states of donors between the emotional and irritant

Table 1. Correlations between all dependent measures in Study 1.

Dependent variable	Condition tears/ saline	Attract. (WB)	SD traits (WF)	SD traits (WB)	F/P mood change	Generosity	Connectedness
Attractiveness – WF	Emotional	.72**	.36	.04	–.24	–.02	–.21
	Irritant	.61**	.67**	.42*	.49*	–.11	.35
	Saline	.84**	.61**	.53**	–.04	.17	.17
Attractiveness – WB	Emotional		.29	.32	.33	–.36	.00
	Irritant		.55**	.67**	.09	–.29	.25
	Saline		.51**	.64**	–.31	.08	.12
Socially desirable traits – WF	Emotional			.67**	.30	.11	.19
	Irritant			.76**	.24	–.23	.36
	Saline			.83**	.31	.28	.02
Socially desirable traits – WB	Emotional				.12	–.16	.32
	Irritant				.24	–.19	.35
	Saline				.04	.27	–.07
Friendly/peaceful mood change	Emotional					–.31	.48*
	Irritant					.16	.39
	Saline					.19	–.13
Generosity	Emotional						–.09
	Irritant						.18
	Saline						–.21

Note: WF, women's faces; WB, women in lingerie or bikini.

* $p < .05$.

** $p < .01$.

tears conditions was observed. As expected, negative emotion was higher during harvesting emotional tears ($M = 26.88$; $SD = 4.52$) than during irritant tears collection ($M = 18.40$; $SD = 1.96$); $t(48) = 8.60$, $p < 0.001$, $d = 2.48$. Correlations between all dependent measures, for each condition separately, are presented in Table 1. Correlations between the attractiveness scores and social-desirability variables were generally large as expected, whereas other correlations were predominantly weaker.

The means and standard deviations of all dependent measures as well as the results of seven one-way ANOVAs with *Group* (emotional tears, irritant tears and saline) as independent variable are presented in Table 2. The results of the t -tests comparing saline and emotional tears condition are presented as well, in order to be able to make a direct comparison with Gelstein et al.'s (2011) results. We found no

significant group differences for any of the dependent measures, that is, the assignment to the three groups did not result in significant differences for any of the assessed variables (all p 's $> .05$). Note also that effects are in both directions, and effect sizes are generally small.

In order to control for the possibility that the answers of certain participants were influenced by specific *response set tendencies*, we repeated the analysis of the effects of the variable *Group* on the variables *Attractiveness* (WF and WB) with socially desirable traits as covariates, separately for the WB and WF pictures. The results remained the same, that is, the three groups did not differ on any of the assessed variables (all p 's $> .05$).

In addition, we controlled for all the variables (friendly and peaceful mood, PA and NA at the beginning of the experiment) that correlated with any of the

Table 2. Means and standard deviations of all dependent measures in Study 1, and the results of ANOVAs and t -tests.

Dependent variable	Emotional tears $N = 25$ $M (SD)$	Irritant tears $N = 25$ $M (SD)$	Saline $N = 25$ $M (SD)$	η_p^2	p (ANOVA)	t	p (t -test)
Attractiveness – WF	7.31 (1.40)	7.41 (1.48)	7.14 (1.20)	.00	.87	–0.47	.64
Attractiveness – WB	7.40 (1.48)	7.35 (1.63)	7.02 (1.61)	.01	.64	–0.88	.39
Socially desirable traits – WF	5.92 (.90)	6.04 (1.19)	5.78 (1.00)	.01	.66	–0.53	.60
Socially desirable traits – WB	5.17 (.91)	5.43 (1.26)	5.19 (1.26)	.01	.67	0.05	.96
Friendly and peaceful mood change	–7.19 (18.8)	–2.31 (18.0)	0.95 (15.7)	.04	.25	–1.68	.10
Generosity	41.0 (19.6)	42.8 (19.0)	47.4 (22.8)	.02	.53	–1.06	.29
Connectedness	4.88 (1.81)	4.52 (1.98)	5.28 (1.70)	.03	.35	–0.81	.42

Note: WF, women's faces; WB, women in lingerie or bikini, η_p^2 , effect size for three groups comparison (ANOVA). t -values refer to comparisons between saline and emotional tears groups only. A negative value for *Friendly and peaceful mood changes* represents a decrease.

independent or dependent variables, and we repeated all the statistical procedures by excluding the six participants that reported suffering from cold sores on their nose, and by controlling for participants' smoking. This also failed to influence the previously reported picture (no significant differences).

In order to evaluate whether there was a relationship between the donors' emotional state during the tear collection and any of the dependent measures, we calculated the inter-correlations among the 25 participants in the irritant and emotional tears conditions. We also repeated the analyses by partialising all the covariates listed above. None of the correlations was significant. Finally, also when all the analyses were repeated by using the single-item attractiveness measure from Gelstein et al. (2011) study, no significant differences between the groups were observed.

Discussion

The current findings failed to provide support for the notion that negative emotional or irritant female tears may contain a substance that decreases perceived sexual attractiveness (and supposedly arousal) in young males, or that affects male behaviour in any of the here proposed theoretically relevant ways. However, the present study can be criticised on an important respect. Given the generally lower statistical power of between-subjects design as compared to within-subjects design, the number of 25 participants in each group makes the statistical power of this study relatively lower in comparison to that of the Gelstein et al. (2011) study. Because of the between-subjects comparison, the inter-individual variation in responses to pictures could overshadow possible intra-individual variation that may be the consequence of sniffing tears.

Study 2

To eliminate the limitation addressed above, Study 2 was conducted on a larger sample of participants (i.e. 150 participants or 50 instead of 25 in each of the three groups). Additionally, it combined a between-subjects design with a within-subjects design in a subsample of 75 participants (25 participants from each of the three groups), allowing us to compare the self-reported attractiveness of female pictures before and after the inhalation of saline and the two types of tears. In Study 2, we also extended our research by including alternative stimuli and

measuring additional theoretically relevant dependent variables. Moreover, Study 2 was conducted in a different country (The Netherlands) than Study 1 (Croatia).

In contrast to Study 1, we measured only the self-reported attractiveness of the whole body pictures and not of the pictures of faces only. For the evaluation of possible effects on the feelings of aggressiveness, we used different measures than those applied in Study 1. More specifically, instead of the friendly mood measure we included (a) a self-report measure of general aggression tendencies and (b) a measure of aggressive behaviour based on the proposed punishment in response to two vignettes describing committed frauds.

Method

Participants and design

One hundred fifty male students (age range 18–29 years, $M = 21.5$; $SD = 2.4$) received course credits (in case of psychology students) or a university cafeteria voucher of €3 for participation. Students suffering from cold sores on their nose (self-report on one item) were excluded from participation. Two samples of 75 participants (Sample 1 and Sample 2) were enrolled in the study, the second sample being tested 18 months after the first one. Each sample underwent a procedure that was different in several details that will be clarified below.

For sample 1 and for the combination of both samples, the same general procedure was used as in Study 1. In addition, sample 2 underwent a procedure based on a mixed within- and between-subjects design. More precisely, before their assignment to one of the three conditions, participants from sample 2 also completed the baseline rating of the comparable set of pictures.

Tears collection and procedure

The tears were harvested from six donors (19–21 years). The period during which the compound was kept in test tubes before the application varied between 5 and 30 minutes (sample 1) or 5 and 45 minutes (sample 2).

In Study 2, we applied the same procedure as in Study 1, with the following modifications. In addition to the rating of pictures following the inhalation of one of the three substances, the participants from

sample 2 also rated the pictures immediately after signing the informed consent, just before the inhalation. The number of times the participants were instructed to sniff deeply the tears (or saline) in the test tube was varied across the two samples, with sniffing for three times (sample 1) or ten times (sample 2). After participants had finished the (final) ratings of the pictures, the *aggressive tendencies* scale (sample 1) and the questions about the punishment (both samples) and connectedness with others (IOS) (sample 1) were presented. All measures were based on paper-and-pencil (sample 1) and computer screen answers (sample 2).

Stimuli

Two sets of 40 full-colour pictures of highly attractive WB were selected by the experimenters. These were rated for attractiveness in two pilot studies (one for each sample) by 10 and 13 male students (age range 18–25) on a 9 points Likert scale. Three sets of seven pictures with the highest attractiveness scores were selected from each pilot study, one set for sample 1 and two sets for sample 2.

Measures

The pictures were rated on the same four attractiveness items that were used in Study 1, on a VAS scale ranging from 0 to 100 (sample 2), and a 9-points scale, which was linearly transformed to a scale from 0 to 100 (sample 1). Cronbach alpha reliabilities for perceived sexual attractiveness ranged from 0.92 (sample 2, pre-inhalation) to 0.94 (sample 2, post-inhalation).

Aggression tendencies were assessed in the following two ways. Firstly, an 11 items measure was developed, to be rated on a 4-point Likert response format (e.g. *If necessary, I can be aggressive; You should never show your fear*, Cronbach alpha = .79). The items were

comparable to those from the *Psychopathic Personality Inventory*, which predicts overt aggressive behaviour (Neumann, Malterer, & Newman, 2008; Patrick, Poythress, Edens, Lilienfeld, & Benning, 2006). Secondly, we presented the participants with two different vignettes describing the case of a man who had committed fraud, and we asked them to decide how severely this man should be *punished*. In previous studies, responses to vignettes were shown to represent a reliable tool for measurement of aggression (e.g. O'Connor, Archer, & Wu, 2001). For sample 1, the punishment was quantified in years of imprisonment. For sample 2, a composite variable was created from responses to eleven items describing different types of aggressive revenge (Cronbach Alpha = .63). *Social Connectedness* was measured in the same way as in Study 1, applying the IOS (Aron et al., 1992).

Results

None of the potentially confounding variables (donors 1–6 and participants' age) were significantly related to any of the dependent variables. Within the sample 1, the correlations between aggressive tendencies and punishment in the emotional tears, irritant tears, and saline conditions were $-.22$, $-.11$, and $.40$, respectively. Between connectedness and aggressive tendencies they were $.20$, $-.40$, and $.03$, and between connectedness and punishment scores $-.03$, $.26$, and $.20$. For sample 2, correlations between attractiveness and punishment were $.31$, $.09$, $.38$, respectively.

Five 3×2 ANOVAs were computed with group (emotional tears, irritant tears & saline) and sample (1 & 2) as independent variables, and the measures of attractiveness (both samples), punishment (each sample separately) and connectedness and aggression (sample 1), as dependent variables. Table 3 displays the means and standard deviations of the dependent measures and the results of the ANOVAs. We did not obtain significant group differences for

Table 3. Means and standard deviations of all dependent measures in the Study 2, and estimates of effect sizes and p -values.

Measure/sample	Emotional tears		Irritant tears		Saline		η_p^2	p ANOVA	t	p t -test
	M (SD)	N	M (SD)	N	M (SD)	N				
Attractiveness, both samples	69.31 (13.69)	50	71.97 (15.85)	50	71.88 (16.46)	49	.01	.62	0.84	0.40
Aggressive tendencies – sample 1	24.5 (4.5)	25	24.7 (5.22)	24	23.6 (4.6)	24	.01	.68	–0.69	.50
Punishment vignette – sample 1	5.8 (3.2)	24	5.8 (2.3)	24	5.4 (3.0)	25	.00	.86	–0.44	.66
Punishment vignette – sample 2	47.50 (17.03)	25	43.83 (12.63)	25	46.47 (12.20)	25	.01	.64	–0.25	.81
Connectedness sample 1	3.6 (1.6)	25	3.7 (1.6)	24	3.8 (1.2)	25	.00	.91	–0.41	.69

Note: η_p^2 , effect size for three groups comparison (ANOVA). t -values refer to comparisons between saline and emotional tears groups only. Three participants from sample 1 were excluded from different analyses due to missing answers on single items.

Table 4. Means and standard deviations of attractiveness ratings for tears and saline conditions in sample 2 before and after inhalation, followed by estimates of effect sizes and p - values.

	Emotional tears M (SD)	Irritant tears M (SD)	Saline M (SD)	η_p^2	p ANOVA	t	p t -test
Attractiveness before inhalation	71.60 (13.55)	69.99 (16.82)	69.13 (18.85)	0.01	.70	0.78	.44
Attractiveness after inhalation	71.53 (14.73)	70.43 (18.40)	70.67 (20.39)				

Note: η_p^2 , effect size of the interaction between group and measurement time. t -values refer to the comparison of change scores of attractiveness between saline and emotional tears groups only.

any of the dependent variables, and effect sizes were small. In other words, the three conditions did not result in different effects on the assessed variables.

The attractiveness scores from sample 2 were additionally analysed by a mixed 3×2 ANOVA, with group (emotional tears, irritant tears and saline) as a between-subjects independent variable and time (before and after inhalation) as a within-subjects independent variable. No significant interaction between group and time on perceived sexual attractiveness was observed (Table 4). Finally, similar as in Study 1, the differences between the groups remained non-significant when all analyses were repeated using the single-item measure of attractiveness.

Discussion

Similar to Study 1, findings of Study 2 failed to provide support for the notion that fresh female tears decrease young males' perception of female attractiveness, as well as for any other behavioural effect of exposure to tears. Although Study 2 was designed in such a way that it had greater statistical power than Study 1 and Gelstein et al.'s (2011) original study, it can be criticised in other aspects. First, contrary to the original Gelstein et al. (2011) study, we did not use pictures of faces in Study 2, but rather whole bodies of specially selected sexually attractive women. The inhalation of tears may only decrease perceived attractiveness when less attractive stimuli are presented, although such a possibility seems not plausible. More importantly, although we did control for the baseline reports of perceived attractiveness of woman on the pictures in a subsample of participants, this procedure could not be regarded as a full within-subjects design because these participants (a) viewed different pictures during each measurement, and (b) they did not inhale any compound before the first measurement. In Study 3, we aimed to conduct a real replication study, with all procedures similar to those used in the original Gelstein et al. (2011) study.

Study 3

In Studies 1 and 2, the hypotheses about the chemosignalling effects of tears on male behaviour were tested using a between-subjects design, or a mixed design based on comparing behaviour before and after the administration of tears or saline. Since the hypothesised effects were not observed in both studies, Study 3 was designed completely as a replication, that is, it was based on a within-subjects design and conducted in the same way as the Gelstein et al. (2011) study. In addition, we measured emotional states of donors while harvesting tears.

Method

Participants and design

Twenty-eight male students (age range 18–34; $M = 22.88$, $SD = 2.92$) received a university cafeteria voucher of €3 for participation. Six participants reported suffering from cold sores on their nose, as assessed by a one-item question. The design was completely the same as the one from the Gelstein et al. (2011) study. Participants were exposed to one of the two compounds (tears or saline) on each of the two occasions when they visited the laboratory.

Stimuli, measures and procedure

Initially, the same 18 pictures as those used in the original Gelstein et al. (2011) study (NimStim database, Tottenham et al., 2009) were selected. All the pictures were rated on attractiveness in the first pilot study by 10 male participants (age range 22–37, $M = 27.8$, $SD = 5.43$), on a 100 points VAS scale. Due to the unexpectedly low ratings of these original pictures ($M = 21.20$, $SD = 17.35$) as compared to the ratings for the saline condition in the original study ($M = 46.3$, $SD = 12.5$), a new selection of 36 pictures was made from the *Karolinska Directed Emotional Faces* database (Lundqvist, Flykt, & Öhman, 1998) and from various internet resources. This selection was rated in the second

pilot study by nine participants (age range 19–37, $M = 25.35$, $SD = 4.18$), where 18 pictures with average ratings of attractiveness ($M = 52.96$, $SD = 12.22$) comparable to the original study were selected. In the main study, the average scores of the ratings of these 18 pictures were used to measure changes in sexual attraction (tears: Cronbach $\alpha = 0.92$) (saline: Cronbach $\alpha = 0.92$). Donors' emotional states were measured with the same scale as in Study 1, that showed satisfactory reliability (Cronbach $\alpha = 0.75$). Since the order of the administration of tears and saline was randomly rotated, 16 participants received tears during the first session and saline during the second, while for the other 12 participants the order was the opposite.

Results

None of the potentially confounding variables (donor 1 or 2, participants' age, suffering from cold sores on the nose) were significantly related to the ratings of sexual attractiveness. Also, the ratings of attractiveness did not significantly correlate with the emotional states of the tears donors during the tears collection. The 2 (order of tears/saline administration) \times 2 (condition – tears/saline) mixed-effects ANOVA showed no effect of the variable order ($F(1, 26) = 0.10$, $p = .92$, $\eta_p^2 = .00$), and no order \times condition interaction ($F(1, 26) = 1.36$, $p = .25$, $\eta_p^2 = .05$). Most importantly, no effect of the condition on the ratings of attractiveness was observed ($F(1, 26) = .04$, $p = .84$, $\eta_p^2 = .00$; saline: $M = 41.66$, $SD = 13.76$; tears: $M = 41.62$, $SD = 13.96$). Excluding the six participants that reported suffering from cold sores on their nose from the analysis did not affect the previously reported picture (no significant differences).

Meta-analysis

Because the absence of a statistically significant effect in each of our studies may be the result of low statistical power, we also carried out a meta-analysis on similar effects, that is, the four ratings of attractiveness (Study 1, Study 2, Study 2b, Study 3, see row 1 in Tables 2 and 3, the complete Table 4, and the results section in Study 3). First, we converted the dependent-sample effect size measure of Study 3 into the same metric (Hedges g) as the other three measures (Viechtbauer, 2007: Eq. 5, 12, 15, 37). Then we conducted a fixed-effect meta-analysis using the metafor package (Viechtbauer, 2010), which in a combined effect size estimate equal to $g = 0.030$ ($z = 0.41$, $p = 0.68$,

CI interval from -0.12 to 0.17). Thus, the meta-analysis confirms that no effect of tears versus saline on the attractiveness ratings exists, and at the same time the lack of statistical power as a plausible explanation for our findings is discarded.

General discussion

In three studies, we attempted to replicate and extend Gelstein et al.'s (2011) observations, which suggested that fresh, sad female tears would have a dampening effect on male's sexual arousal (in a non-erotic context). However, our three studies separately and combined in a meta-analysis did not reproduce their findings. Even though we used larger samples of participants than Gelstein et al., we did not obtain any statistically significant difference between the effects of exposure to sad tears, irritant tears, and saline on any of the three dependent variables related to sexual attractiveness, even when taking into account several possible confounders. Similarly, no effects of inhaling (both emotional and irritant) tears were observed on any of the four additional, theoretically relevant dependent variables.

A major strength of Gelstein et al.'s (2011) study was that the effects were found in three independent groups and on different indicators of (sexual) arousal (self-reports of sexual attraction and arousal, peripheral physiology and brain activity), yielding a rather consistent pattern of findings, whereas no effects were found on the indicators of empathy. One may further challenge Gelstein et al.'s assumption that the observed mean decrease in saliva testosterone from 152 pg/l to 133 pg/l does have a substantial effect on sexual arousal, given Mazur and Booth's (1998) conclusion that fluctuations in this hormone do not have any observable effect on sexual behaviour, if they are within the normal range and if a minimal amount of this hormone is present in the blood. Furthermore, in none of their three studies, subjective and objective (sexual) arousal were assessed simultaneously, which prevents the opportunity to evaluate directly the implicitly assumed relationships. In contrast, several different but theoretically interlinked responses were measured in parallel in each of our first two studies. A final already noted remarkable characteristic of the Gelstein et al.'s study is that they did not assess effects after explicit sexual stimulation. It is unclear why they measured perception of sexual attractiveness and arousal during exposure to neutral, happy or even sad stimuli, when the anticipated levels of sexual

arousal may be expected to be minimal, rather than after exposure to explicit erotic stimuli. In our three studies, the previous results were not replicated neither with moderately attractive nor with highly sexually attractive stimuli. For future studies, we thus recommend simultaneous multimodal assessment in response to both explicitly sexual and non-sexual stimulation.

When trying to explain their results, Gelstein et al. (2011) avoided the term pheromones and rather talked about chemosignals. However, the suggested function of tears certainly implies the involvement of chemical signals used to communicate with conspecifics or to trigger specific behaviour and physiological responses, which corresponds to the definition of pheromones (Karlson & Luscher, 1959). Therefore, if tears would exert additional signalling effects via the chemical channel, the transmitted substances should also be labelled as pheromones. In mammals, pheromones serve several functions, although they seem to operate mainly in the domain of alarm signalling and increasing, rather than decreasing, sexual arousal or aggressiveness (Tirindelli, DiBattista, Pifferi, & Menini, 2009). The evidence that these substances also reduce sexual drive and aggression in mammals is very limited (however, see Ferrero et al., 2013 for an exception). In primates, pheromones are much less present than in other mammalian taxa (Tirindelli et al., 2009). And when it comes to humans, the state-of-knowledge with respect to the role of pheromones is not consistent, and there is substantial disagreement among scientists in this field even in the most general issues. Despite the numerous claims about their existence (e.g. Havlicek, Murray, Saxton, & Roberts, 2010), it is still not known whether humans have pheromones at all (Wyatt, 2015; for a review see Wysocki & Preti, 2004). If they do, currently the most convincing evidence for their existence comes from the research on sucking and nipple search behaviours of infants, rather than from research on adult sexual behaviour (Wyatt, 2015).

Various pheromones in mammals are emitted from perianal regions, skin and vaginal glands, or from unique organs such as the temporal gland in elephants. They are typically contained in body fluids, such as sweat, urine, mucous secretions of genitals, saliva and breath (for reviews see Tirindelli et al., 2009; Verheggen, Haubruge, & Mescher, 2010; Wyatt, 2015). Only in certain types of rodents, pheromones are also secreted from the *extraorbital lacrimal glands* (Ferrero et al., 2013; Kimoto, Haga, Sato, & Touhara, 2005; Kimoto et al.,

2007; Thompson, Napier, & Wekesa, 2007), which may be phylogenetically related to the lacrimal glands in humans. Indeed, this was one of the reasons for Gelstein et al. (2011) to assume that a comparable mechanism may operate in humans. However, the human genome fails to contain information that is needed for the synthesis of the *exocrine gland-secreting peptides* secreted from the above-mentioned lacrimal glands in rodents (Tirindelli et al., 2009). In addition, in rodents these signalling molecules are sensed by the *vomeroneasal organ* (VMO), a structure located in the nose and with rich connections to the brain, whose function is to detect pheromones. However, adult humans no longer possess that organ (Wyatt, 2015; Wysocki & Preti, 2004), which additionally decreases the probability of any phylogenetic link between human chemosignals and this specific type of pheromonal communication in rodents. Alternatively, assuming that human tears contain pheromones that can be perceived and consequently affect conspecifics, one may expect that the specific state of the donor, when she produces the tears, is also important. Perhaps tears produce the effects reported in Gelstein et al. studies only during the fertile phase of menstrual cycle, in order to prevent the conception of a child, when a woman's well-being is compromised and, consequently, she is less able to raise successfully her offspring (Silver, 2011).

Although we aimed to replicate Gelstein et al. (2011) as closely as possible, each of our three studies had some specific deviant features as well. In particular, this concerned the design and the stimuli used in our first two studies. Gelstein et al. used a repeated measures design, whereas we compared different groups of participants, which might have increased the error variance. However, it is reasonable to assume that in our additional analyses, in which we statistically controlled for the effects of tears/saline on theoretically irrelevant ratings of women, we partly excluded not just the possibility that stable individual differences might have influenced the results (the main weakness of our approach), but also the possibility that some intra-individual variations might have influenced the results (the possible weakness of a within-subjects design). Moreover, the chemosignalling effects of tears were neither replicated in our third study, although the within-subjects design was the same as the original one, by which the above-discussed limitation was removed.

An additional asset of the studies reported in this paper is the usage of five different subsets of stimuli,

allowing us to increase the generalisability of the results (see also Westfall, Kenny, & Judd, 2014). In addition, we used both the one-item measure from Gelstein et al. (2011) and the overlapping four-item composite measure of attractiveness, which allowed us to cover more aspects of sexual attraction, without losing the possibility of direct comparison of previous and new results. Regarding the stimuli, whereas Gelstein et al. used pictures depicting only the faces of average women, we used both the pictures of averagely and highly attractive WF as well as pictures of semi-nude women. We anticipated that the use of sexually arousing stimuli would have increased our chances of finding negative influences. However, alternatively, it could be argued that the aroused sexual drive was so strong that it could easily resist weak inhibitory influences. The latter reasoning seems less likely, since the effects were not replicated, neither for the pictures of semi-nude women nor those of WF, even in Study 3 in which the pre-selected pictures of the averagely attractive faces were used.

The rejection of the main hypothesis based on the results of the original Gelstein et al. (2011) study is not to be considered surprising given the recently emerging arguments that false findings may represent the majority of the published psychological research (see Open Science Collaboration, 2015). Similar problems are omnipresent in the specific field of research on the possible existence of pheromones in humans (Wyatt, 2015). Being aware that our studies do not provide a definitive answer, we nevertheless feel that there is now sufficient reason to conclude that, if there is any substance in female's tears that has a dampening effect on the sexual arousal of males, this influence is very modest at best and certainly does not always impact every male in his sexual functioning. Crying thus seems to exert its powerful effect on others more likely by its acoustical and visual aspects than via a chemosensory pathway.

Note

1. The appropriate interaction test is $t = (M_{\text{base,tears}} - M_{\text{sad,tears}} - M_{\text{base,saline}} + M_{\text{sas,saline}}) / SE$, with SE^2 approximated $SE^2_{\text{base,tears}} + SE^2_{\text{sad,tears}} + SE^2_{\text{base,saline}} + SE^2_{\text{sad,saline}} - 2r \times (SE_{\text{base,tears}} \times SE_{\text{sad,tears}} + SE_{\text{base,tears}} \times SE_{\text{base,saline}} + SE_{\text{sad,tears}} \times SE_{\text{sad,saline}} + SE_{\text{base,saline}} \times SE_{\text{sad,tears}}) + 2r \times (SE_{\text{base,tears}} \times SE_{\text{sad,tears}})$. The standard errors and means can be directly computed from Gelstein et al. The correlation r is approximated by the average of the correlations in the tears ($r = .87$) and

saline ($r = .79$) conditions, which can be computed from the reported statistics in both conditions.

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